

Laboratory Manual 2nd Ed., Sambrook, Fritsch and Maniatis, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989.

Example 1. Preparation of recombinant plasmids containing the transcriptional unit encoding JEV prM and E antigens. Genomic RNA was extracted from 150  $\mu$ L of JEV strain SA 14 virus seed grown from mouse brain using a QIAamp™ Viral RNA Kit (Qiagen, Santa Clarita, CA). RNA, adsorbed on a silica membrane, was eluted in 80  $\mu$ L of nuclease-free water, and used as a template for the amplification of JEV prM and E gene coding sequences. Primer sequences were obtained from the work of Nitayaphan et al. (1990). A single cDNA fragment containing the genomic nucleotide region 389-2478 was amplified by the reverse transcriptase-polymerase chain reaction (RT-PCR). Restriction sites KpnI and XbaI, the consensus Kozak ribosomal binding sequence, and the translation initiation site were engineered at the 5' terminus of the cDNA by ampimer 14DV389 (SEQ ID NO:1). An in-frame translation termination codon, followed by a NotI restriction site, was introduced at the 3' terminus of the cDNA by ampimer c14DV2453 (SEQ ID NO:2) (see Figure 2). One-tube RT-PCR was performed using a Titan RT-PCR Kit (Boehringer Mannheim, Indianapolis, IN). 10  $\mu$ L of viral RNA was mixed with 1  $\mu$ L each of 14DV389 (50  $\mu$ M) and c14DV2453 (50  $\mu$ M) and 18  $\mu$ L of nuclease-free water and the mixture was heated at 85°C for 5 min and then cooled to 4°C. 75  $\mu$ L of reaction mix [20  $\mu$ L 5x buffer, 2  $\mu$ L of dNTP mixture (10 mM each), 5  $\mu$ L of dithiothreitol (0.1 mM), 0.5  $\mu$ L of RNasin™ (40 U/ $\mu$ L, 15  
Boehringer Mannheim), 2  $\mu$ L of polymerase mixture, and 45.5  $\mu$ L of nuclease-free water] was added and RT-PCR performed as follows: 1 cycle (50°C for 30 min, 94°C for 3 min, 50°C for 30 s, 68°C for 2.5 min), 9 cycles (94°C for 30 s, 50°C for 30 s, 68°C for 2.5 min), 20 cycles (94°C for 30 s, 50°C for 30 s, 68°C for 2.5 min in the first cycle, with an increment of 5 s per cycle thereafter), and a final extension at 68°C for 15 min. The RT-PCR product was purified by a QIAquick™ PCR Purification Kit (Qiagen) and eluted with 50  $\mu$ L of 1 mM Tris-HCl, pH 7.5.

All vector constructions and analyses were carried out by using standard techniques (Sambrook et al., 1989). RT-PCR amplified cDNA, digested with KpnI and

a specified gene, a control sequence effectively controls expression of the specified gene.

As used herein, a "promoter" is a nucleotide sequence in a nucleic acid TU which serves as a control sequence.

5 As used herein, a "terminator" is an extended nucleotide sequence which acts to induce polyadenylation at the 3' end of a mature mRNA. A terminator sequence is found after, or downstream from, a particular coding sequence.

As used herein, a "host cell" is a prokaryotic or eukaryotic cell harboring a nucleic acid TU coding for one or more gene products, or into which such a TU has been introduced. Thus a host cell harbors a foreign or heterologous substance, the TU, which is not naturally or indigenously found in it as a component. A suitable host cell is one which has the capability for the biosynthesis of the gene products as a consequence of the introduction of the TU. In particular, a suitable host cell is one which responds to a control sequence and to a terminator sequence, if any, that may be included within the TU. In important embodiments of the present invention, the host cell is a mammalian cell. In particularly important embodiments of this invention, the host cell is a naturally occurring cell in the body of a human or nonhuman subject to whom (which) the TU has been administered as a component of a vaccine. Alternatively, in analytical, or diagnostic applications, or for demonstrative purposes, the mammalian cell may be a human or nonhuman cell cultured in vitro.

As used herein, a "vaccine" or a "composition for vaccinating a subject" specific for a particular pathogen relates to a preparation, which, when administered to a subject, leads to an immunogenic response in a subject. As used herein, an "immunogenic" response is one that confers upon the subject protective immunity against the pathogen. Without wishing to be bound by theory, it is believed that an immunogenic response may arise from the generation of neutralizing antibodies, or from cytotoxic cells of the immune system, or both. As used herein, an "immunogenic antigen" is an antigen which leads to an immunogenic response when it is introduced into a subject, or, as in the case of the present invention, when it is synthesized within the cells of a host or a subject. As used herein, an "effective amount" of a vaccine or